

Review Paper

Carcass *Salmonella* and Its Drug Resistance

ABSTRACT

Salmonella are the major pathogenic bacteria in humans as well as in animals. *Salmonella* species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries. Isolation of *Salmonella* from a wide range of sources suggests that *Salmonella* is widespread in food animals and meat products and underlines the necessity for a joint and coordinated surveillance and monitoring programs for salmonellosis and other major food borne zoonotic diseases. Food animals harbor a wide range of *Salmonella* and so act as sources of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis. Salmonellosis is more aggravated by the ever increasing rate of antimicrobial resistance strains in food animals. The high prevalence and dissemination of multidrug resistant (MDR) *Salmonella* have become a growing public health concern. Multidrug resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multidrug resistance have increased considerably in recent years. Food animal consumption is a potential cause for antimicrobial resistant *Salmonella* illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor personal hygiene. So, this review used for updating information on their prevalence and resistance patterns is very important to suggest the acceptance of the carcass in relation to the standards and for proper selection and use of antimicrobial agents in a setting.

Key words; *Salmonella*, Drug resistance, Food animal, Prevalence, Multi-drug resistance

1. INTRODUCTION

There have been heightened concerns about the safety of food animal, not only amongst scientists with an interest in toxicology or microbiology but also economists and other social scientists that focus on the wider socio-economic associated with the safety of a country's food animal supply [1]. *Salmonella* are the major pathogenic bacteria in humans as well as in animals. *Salmonella* species are leading causes of acute gastroenteritis in several countries and salmonellosis remains an important public health problem worldwide, particularly in the developing countries [2]. It is also one of the most common food borne zoonotic diseases. The presence of *Salmonella* in food animals at slaughter and the consequent cross-contamination of edible carcass tissues present a significant food safety hazard [3; 4]. Non-typhoidal *Salmonella* represents an important human and animal pathogen worldwide [5]. Infection in animals is of importance because of the direct economic effect and even greater importance is that animals constitute a vast reservoir of these organisms for human infection [6].

Isolation of *Salmonella* from a wide range of sources suggests that *Salmonella* is widespread in food animals and meat products and underlines the necessity for a joint and coordinated surveillance and monitoring programs for salmonellosis and other major food borne zoonotic diseases. A periodic surveillance of the sources, distribution and prevalent *Salmonella* serotypes in slaughtered food animals, retail meat products and environment is necessary to control the spread of the pathogen and infection of man through contaminated animal products [7]. Often, infected animals shed *Salmonella* in feces without showing clinical signs. Various stress factors such as those associated with transport of animals from farm to slaughterhouse augments shedding of *Salmonella* from carrier animals. Food animals such as cattle may carry *Salmonella* at slaughter and can serve as sources of contamination and provides an opportunity for entry of the pathogen into the food products [8; 9]. This implies that the presence of *Salmonella* in slaughter cattle and slaughterhouse environment and the potential cross-contamination of carcasses and edible organs can pose food safety hazards [9].

Food animals harbor a wide range of *Salmonella* and so act as sources of contamination, which is of paramount epidemiological importance in non-typhoid human salmonellosis. The process of removing the gastrointestinal tract during slaughtering of food animals is regarded as one of the most important sources of carcass and organ contamination. *Salmonella* at slaughterhouse. Moreover, contamination of meat by *Salmonella* may occur at slaughterhouse from

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excretion of symptomless animals, contaminated slaughterhouse equipment, floors and personnel and the pathogen can gain access to meat at any stage during butchering. Cross contamination of carcasses and meat products could continue during subsequent handling, processing, preparation and distribution [10; 11].

Salmonellosis is more aggravated by the ever increasing rate of antimicrobial resistance strains in food animals [2]. The high prevalence and dissemination of multidrug resistant (MDR) *Salmonella* have become a growing public health concern. Of particular significance is the increasing number of *Salmonella* isolates that are resistant to clinically important antimicrobial agents such as fluoroquinolones and third-generation cephalosporins, which are used for the treatment of life threatening disease conditions in humans [12; 9]. Antimicrobial resistant *Salmonella* are increasing due to the use of antimicrobial agents in food animals at sub-therapeutic level or prophylactic doses which may promote on farm selection of antimicrobial resistant strains and markedly increase the human health risks associated with consumption of contaminated meat products [7; 13; 2]. Antimicrobial resistant *Salmonella* and other zoonotic bacterial pathogens can be transferred from animals to humans through consumption of contaminated food and food products and thus present a public health risk. The increase in *Salmonella* resistance to the commonly used antimicrobials both in the public health and veterinary sectors is one of the major threats of health care worldwide [7]. Cattle have been implicated as a source of human infection with antimicrobial resistant *Salmonella* through direct contact with livestock and through the isolation of antimicrobial resistant *Salmonella* from raw milk, cheddar cheese, and hamburger meat [2].

The emergence and spread of antimicrobial resistant *Salmonella* strains in food animals and humans may be associated with the use of medicated feeds in intensive animal husbandry systems, sub therapeutic doses and indiscriminate uses of antimicrobials both in animal and human treatments. Various antimicrobials in intensively managed food animals including chicken are often administered through the feed or drinking water either for therapy, prophylaxis or growth promotion. This enhances the risk of proliferation of resistant strains, which can have severe consequences on human health [7].

Multidrug resistant (MDR) strains of *Salmonella* are now encountered frequently and the rates of multidrug resistance have increased considerably in recent years. Even worse, some variants of *Salmonella* have developed multidrug resistance as an integral part of the genetic material of the organism, and are therefore likely to retain their drug resistant genes even when antimicrobial drugs are no longer used (14). Most of the strains of *Salmonella* Typhimurium isolated in a study in western part of Nigeria were resistance to drugs like streptomycin, amoxicillin, tetracycline, ampicillin, kanamycin and chloramphenicol. This data is alarming since the isolates were already showing high resistance to drugs that are meant as alternate therapy to salmonellosis treatment; especially isolates from blood were resistance to the commonly used antibiotics. Drug resistant *Salmonella* emerged in response to antimicrobial usage in food animals, which has also contributed or resulted in major outbreaks of salmonellosis. Selective pressure from the use of antimicrobials is a major driving force behind the emergence of resistance, but other factors also need to be taken into consideration [15].

Food animal consumption is a potential cause for antimicrobial resistant *Salmonella* illnesses besides, the common factors such as overcrowding, poverty, inadequate sanitary conditions, and poor personal hygiene [16]. Because *Salmonella* contamination was high in food items such as minced beef, mutton and pork samples obtained from retail supermarkets and slaughterhouse, that means *Salmonella* contamination is especially high in meat samples as compared to others food items. Supermarket and slaughterhouse personnel are also a victim of *Salmonella* contamination and the magnitude of the problem represents a real public health hazard [17; 18].

Problems have their origin in the methods of farming of animal foods. Many farmers are illiterate and follow methods of production that are centuries old. They live in very close contact with their animals, often under poor hygienic conditions, thereby increasing the likelihood of food borne zoonoses. However, considerable proportion of patients may not visit health centers unless symptoms are serious due to shortage of resources and lack of awareness. So, this review used for updating information on their prevalence and resistance patterns which is very important to suggest the acceptance of the carcass in relation to the standards and for proper selection and use of antimicrobial agents.

2. SALMONELLOSIS

2.1. *Salmonella* in cattle

Food borne sources of *Salmonella* include a wide range of domestic and wild animals and a variety of foodstuffs including food of both animal and plant origin [19]. *Salmonella* serotypes have a broad host range [20], prevalent in the warm blooded animal population [21], including rodents [22], snakes [23], and free living terrestrial and aquatic turtles [24]. *Salmonella* Typhimurium and *Salmonella* Dublin appear to be the commonest serovars isolated from cattle, although the distribution of these two serovars may differ between countries, and *S. Dublin* is thought not to be present in some countries [25]. Infection by this *Salmonella* serovars occurs when susceptible cattle ingest feed or water that has been contaminated with feces from animals shedding the organism. Some adult cattle which recover from *Salmonella* infection, especially in the case of *S. Dublin*, may become active carriers and excrete the organism continuously or intermittently in their feces for years. Salmonellosis has a wide spectrum of manifestations in cattle. Asymptomatic, mild clinical or fulminant bacteremia/septicemia and endotoxemic infections can occur. The number of *Salmonella* required to produce clinical disease is dependent on the virulence of the serotype, infectious dose and immunity of the host. Infection with a host adapted *Salmonella* strain (*S. dublin* in cattle) can result in a cyclic, endemic disease that is maintained on a farm by carrier animals shedding in the feces. The carriers can shed constantly or intermittently [26].

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Members of the genus *Salmonella* pose a serious threat to the domestic food animal including cattle. All animals including cattle are at increased risk of developing disease if their normal flora is disrupted (stress, antibiotics). These circumstances render cattle susceptible to exogenous exposure or activation of silent infections. Poor sanitation, overcrowding, unfavorable weather, stress and surgery, parturition, parasitism, transportation, and concurrent viral infections are all factors which predispose cattle to clinical salmonellosis. In the subclinical form, cattle and other animal may have a latent infection and harbor the pathogen in its lymph nodes, or it may be a carrier and eliminate the agent in its fecal material briefly, intermittently, or persistently [27]. These organisms are responsible for significant morbidity and mortality in their respective hosts, as well as causing substantial disease to humans consuming processed meats derived from the infected cattle. What may be considered now is the emergence of different food vehicles, such as meat, as the source of these infections, forcing industry to examine its management practices and incorporate new procedures to reduce the incidence and severity of the problem [17].

The infectious dose for healthy adult cattle is approximately 10^{9-11} *Salmonella*. In adult cattle salmonellosis commonly occurs close to parturition and may be associated with inter-current disease. The growth of *Salmonella* in the rumen following ingestion is influenced by dietary intake before and after the *Salmonella* is ingested. *Salmonella* disappear rapidly from the rumen of regularly fed cattle, but maintain or increase their numbers when feed intake is decreased or interrupted for one or more days. Feeding after a period of starvation is associated with multiplication of *Salmonella*. Disruption of normal fermentation with production of lactate favors the less fastidious *Salmonella*, which multiplies rapidly using the available substrate. Qualitative dietary stress and dietary changes have been implicated as a predisposing risk factor in *Salmonella* outbreaks in dairy cattle. Reduction in the prevalence of *Salmonella* may be observed following manipulation of the ration formulation and adjustment of feeding practices [28].

Salmonella within and between herd prevalence estimates vary considerably, with between herd point prevalence estimates for cattle operations ranging from 2-42% and within herd estimates for these operations ranging from 0-37% [29; 30]. In addition, herds with clinically sick animals are generally characterized by higher within herd prevalence than herds where clinical salmonellosis is absent, and *Salmonella* distribution may differ between herds with and without clinical cases. Large herd size represents an important risk factor for salmonellosis, and the risk of *Salmonella* shedding seems to vary by production system, housing type, general hygiene level, management type and animal age, although the results reported in the literature have been somewhat contradictory [31]. Calves, heifers, and parturient cows generally appear to be at a particular risk of infection, and one study found heifers and parturient cows to be the most likely cattle to become asymptomatic carriers. The distribution of *Salmonella* among cattle varies greatly over time, and differs among geographic regions, age groups, clinical manifestation, and production systems [32; 30].

Illness from salmonellosis in the cattle is seen predominantly in young calves, although occasionally it is seen in adult cattle as well. *Salmonella* have been isolated from the feces of healthy cattle, where the pathogen may exist as a normal member of the gastrointestinal population or as a transient member of the gastrointestinal microbial population. Researchers have shown that as herd size increased, fecal shedding of *Salmonella* increased. However, other studies have found that herd size did not play a role in *Salmonella* shedding [33]. Genetics plays a very important role in the relationship between *Salmonella* and its potential host animal including cattle. Some *Salmonella* isolates display a very narrow host specificity, while many of the remaining members of this genus express a wider ranging host infectivity. Furthermore, members of a particular *Salmonella* isolate express differential capabilities for infecting a particular host. Conversely, genetics plays an important role in enabling the host to resist infection by a *Salmonella* pathogen [25].

2.2. Mode of transmission of *Salmonella* in cattle

The feco-oral route is the most important mode of transmission of *Salmonella* in animals. Infection in cattle may also occur via other routes, including the respiratory tract, by inhalation of aerosol [34]. First, environment contaminated with *Salmonella* serves as the infection source because *Salmonella* can survive in the environment for a long time. After that, *Salmonella* is transmitted to vectors such as rats, flies and birds where *Salmonella* can shed in their feces for weeks and even months. Following the direct transmission, moving animals such as swine, cattle and chickens act as the important risk factor for infection. These animal reservoirs are infected orally because *Salmonella* normally originates from the contaminated environment and also contaminated feed. Human get infected when eating the food or drinking the water that is contaminated with *Salmonella* through animal reservoirs. However, *Salmonella* Typhi and *Salmonella* Paratyphi A do not have animal reservoir, therefore infection can be happened by eating the improperly handled food by infected individuals [35; 24].

Besides, transmission of *Salmonella* to the food processing plants and equipments for food preparation are also of great importance. The main reservoirs for non-typhoidal *Salmonella* are animals including; poultry, livestock, pets and reptiles [36]. Historically, the major mode of transmission for non-typhoidal *Salmonella* was consumption of inadequately cooked or pasteurized foods of animal origin, such as poultry, beef (including ground beef), fish, eggs, and dairy products (including ice cream) [37]. Once carried by vectors or transferred to food, consumption by human can result in the risk of salmonellosis. The *Salmonella* cells can attach to food contact surfaces such as plastic cutting board which may develop into biofilm once attached and hence cause cross-contamination. Consequently, *Salmonella* can enter the food chain at

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any point from livestock feed, through food manufacturing, processing and retailing as well as catering and food preparation in the home [34].

Infected food handlers have been shown to transmit *Salmonella* and have been responsible for outbreaks. Workers who have been ill can shed *Salmonella* for a median of 30 days (range, 2 days to 280 days). Therefore, assessment of food-worker infection is essential for controlling outbreaks traced to restaurants [38]. As many as 90% of reptiles may be *Salmonella* carriers. Between 3% and 5% of all cases of salmonellosis in humans have been associated with exposure to exotic pets, especially reptiles (including pet turtles, iguanas, lizards, and snakes) [39]. The United States banned the sale of small turtles (carapace < 4 in. [10.2 cm]) in 1975, and reissued a warning because of a resurgence of turtle sales and subsequent outbreaks [40]. Pet rodents probably represent an under-recognized source of human *Salmonella* infection. In 2007, these animals were responsible for an outbreak of multidrug-resistant *Salmonella* in several states. Of 22 patients interviewed, 13 (59%) in 10 states reported exposure to pet hamsters, rats, or mice, and 2 (9%) had secondary infections [41]. Animals in petting zoos may also serve as sources of infection, as also certain other animals, such as baby poultry and livestock. Other modes of transmission include ingestion of contaminated water and contact with contaminated dyes and medical instruments [37].

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2.3. Sources of contamination and microbial load in cattle carcass

The hygiene conditions at the production line for slaughter animals are one of the critical factors influencing both, the level of carcass microbial contamination and the type of determined microorganisms. Experimental studies have shown that total aerobic bacterial contamination depends on a slaughter site and may range in bovine carcasses from $10^2/\text{cm}^2$ up to $10^6/\text{cm}^2$ [42]. Effective intervention to reduce contamination of beef carcasses begins with determining potential sources of contamination. Tissues under the hide of healthy cattle are usually sterile [43]. Consequently, tissues become contaminated during the slaughtering process. Sources of meat contamination during slaughter may be classified as handling practices of slaughter man and cross-contamination. The extent to which potential contamination sources become hazardous to public health depends on management and unpredictable events or factors. Even in the best managed slaughter facilities, contamination may still occur. Fortunately, most bacterial colonies which have been isolated from beef carcasses have been non-pathogenic, although human pathogens such as *Salmonella* have been isolated also [44].

Surface contamination of carcasses during slaughter and processing can be reduced by ensuring good manufacturing practices such as hygiene and sanitation of the floor, equipment, and carcasses, with suitable disinfectants and sanitizers [45]. Meat has a microbial flora from different sources. Also, several methods have been proposed for decreasing the microbial flora to a standard allowance for increasing the shelf-life and decontamination of microbial pathogens including cooking, freezing, fermenting, salting, smoking, drying, and pickling [46].

A cross-sectional study was conducted in cattle and pig carcasses showed that mean total viable counts ranged from 2.4 to 4.2 $\log_{10}\text{cfu}/\text{cm}^2$ on pig carcasses and from 2.7 to 3.8 $\log_{10}\text{cfu}/\text{cm}^2$ on cattle carcasses. Amongst sites, the back (pigs) and neck (cattle) tended to yield higher total viable counts [47]. Similarly in calf carcasses results have shown that the total aerobic bacteria count in each slaughter stage ranged from $3.5 \times 10^3\text{cfu}/\text{cm}^2$ up to $7.0 \times 10^3\text{cfu}/\text{cm}^2$. In most cases, no significant differences of total bacterial contamination of carcasses in each slaughter stage were obtained. At stage II, a significantly higher total aerobic bacteria count ($10^4\text{cfu}/\text{cm}^2$) was observed, when compared to stage I where $2.3 \times 10^3\text{cfu}/\text{cm}^2$ was reached [42]. In Khartoum State the study was conducted to evaluate the bacteriological contamination in indigenous cattle carcasses in slaughterhouse, during April 2008- June 2008. The mean total viable count of bacteria after skinning, evisceration and washing operations at shoulder site were, 3.03 ± 0.15 , 2.73 ± 0.02 and 2.79 ± 0.10 $\log_{10}\text{cfu}/\text{cm}^2$, in the neck site were 3.65 ± 0.02 , 3.42 ± 0.02 and 3.72 ± 0.02 $\log_{10}\text{cfu}/\text{cm}^2$ and in brisket site were 3.1 ± 0.14 , 3.71 ± 0.04 and 3.65 ± 0.02 , respectively. In addition, in the rump site, the total viable counts in these operations were 3.24 ± 0.02 , 2.88 ± 0.02 , and 3.18 ± 0.03 $\log_{10}\text{cfu}/\text{cm}^2$ in three points of operation [48]. Another study in China showed that beef samples from Sakasaka had the highest mean total bacterial count of $1.67 \times 10^6\text{cfu}/\text{cm}^2$, followed by Aboabo ($5.75 \times 10^5\text{cfu}/\text{cm}^2$), Central Market (internal) ($4.325 \times 10^5\text{cfu}/\text{cm}^2$), Nyohini ($3.875 \times 10^5\text{cfu}/\text{cm}^2$) and Central Market (external) ($4.325 \times 10^5\text{cfu}/\text{cm}^2$). While their mean log counts were 6.22, 5.76, 5.64, 5.59 and 5.57 for Sakasaka, Aboabo, Central Market (internal), Nyohini and Central Market (external), respectively [49]. In Mumbai, a total of 54 swab samples were from the abattoir, while 81 swab samples were from three meat shops reported that the average total viable count (TVC) for all environmental contamination points in the abattoir was 5.80 ± 0.17 , where as in the shops it was 6.05 ± 0.25 $\log_{10}\text{cfu}/\text{cm}^2$ indicating higher microbial load in traditional meat shops [50].

2.7. Predisposing risk factors for the prevalence of *Salmonella* in slaughtered cattle carcass

There is a lack of studies of risk factors for *Salmonella* in cattle [51]. The risk factor was divided into three sections: (i) slaughterhouse practices (cleaning and disinfection of pens, truck washing, frequency of knife disinfection, water treatment, etc.); (ii) information on the animal lots (time from farm to slaughter, cleanliness of the animals, tattoo number, and producer number); and (iii) any event during the slaughtering that may have affected the contamination of carcasses (mechanical problems, slaughter rate, stops, condemnation rate, contamination rate, gut ruptures, percentage of filled stomachs, and employee training) [52].

The probability that a live animal is contaminated (both internally and externally) and the extent of contamination (pathogen load) depend on factors which can be affected by management before transport to the abattoir (on-farm and market factors), during transport, and while the animals are being held at the abattoir before slaughter [44]. However, suggestions of factors of importance for *Salmonella* occurrence in cattle generally include hygienic factors in the herds e.g. flies in pens [51] contact with poultry manure or wild bird manure, outdoor calving, herd size and herd expansions [53]. Hygiene and contacts at markets and in vehicles are also likely to be important risk factors before slaughter. In Danish dairy herds, risk factors for becoming infected in 2003 included herd size, number of purchased cattle from test-positive herds and number of test-positive neighbor herds. Organic herds were less likely to recover than conventional herds indicating that different types of management can influence the occurrence of *Salmonella* in cattle herds [54].

Salmonella contaminated carcass could be from the actual infection of food animals at the farm. Off-farm rearing of heifers has been acknowledged as an important risk of infection with multi-drug-resistant *Salmonella* in US dairy herds [49]. One study also reported that for heifers and cows, recent antimicrobial treatment increased the probability of isolating *Salmonella* from fecal samples. Also, *Salmonella* has been associated with high calf mortality in dairy herds. This may be due to both direct effects of the infection and underlying management factors [32].

Transport factors such as the type and cleanliness of transport conveyance, distance travelled and duration of journey, harshness of ride, density of animals in the conveyance and frequency of stops, may affect the pathogen load including *Salmonella*. Interruption of feeding just before transport, during transport, and while being held at auction barns and abattoirs affect the growth of potential pathogens in the rumen and fecal shedding of bacteria. The number of calves which shed *Salmonella* has been found to increase after transportation [55]. The length of time animals are held at the abattoir before slaughter can affect the pathogen load by increasing the probability of exposure and infection. Sanitation of walkways, pen floors, railings, feed and water affect the pathogen load. Steep walkways with sharp turns increase the likelihood that animals will fall and become contaminated or injured. Excessive prodding of animals to move them bruises tissue [44].

Reptiles also the risk factors for cattle contamination by *Salmonella*. *S. enterica* subspecies *arizonae* is widely distributed in reptilian species. Reptiles, particularly snakes, are the natural reservoirs of *S. enterica* subspecies *arizonae*. This organism has also been responsible for severe outbreaks in turkeys, chickens and sheep and cattle [56]. Though the organism is rare, several studies suggest that snakes and reptiles harbor it and transmit it to humans and other mammals, resulting in gastroenteritis and systemic infections [57]. In particular, rattlesnake meat, capsules and powders have been linked to infection with *S. arizonae*, although other animals or animal products have been implicated, including reptiles, poultry, sheep, rats, dogs, and cats [58].

With regard to risk factors at the slaughterhouse associated with the presence of *Salmonella* in the final product, study in Canada demonstrated the importance of the pre-slaughter and pre-evisceration environment on the final status of carcasses. Namely, the cleanliness of the hogs and the status of the scald water proved to be significant factors associated with the final bacteriological status of the carcasses. Results obtained by genetic characterization and serology indicated that particular attention should be paid to the herd contamination levels of incoming animals and the pre-evisceration environment to better control *Salmonella* at slaughter [52]. The feces and fecal contaminated products of animals can contain many enteric organisms including *Salmonella*. When the carcass is opened and the viscera removed, spillage of rumen and intestinal fluids may contaminate the carcass, workers, processing utensils and viscera tables or trucks [44]. People working in meat processing plants also can act as vector of many food borne pathogenic bacteria including *Salmonella* [59]. Microbial contamination of slaughtered cattle carcass results from starts during slaughter, processing and when the carcass becomes contaminated with microorganisms residing on external surfaces of the animal itself, the gastrointestinal tract, lymph nodes of the animal and in the plant environment [60; 61].

Furthermore, certain processing steps increase contamination by spreading the existing contaminants attached to the fresh meat surface to its entire mass or by introducing additional contaminants. For example, meat chopping or grinding results in greater microbial loads because of larger areas of exposed surface, more readily available water and nutrients, additional processing time, and contact with more sources of contamination such as equipment [62]. *Salmonella* contaminated carcass could be from cross-contamination during slaughtering, distribution and subsequent handling and processing. Cross-contamination may arise from knives and hand of the slaughter man. The other probable source of cross-contamination could be from *Salmonella* carrier slaughter house personnel [63]. The study conducted in Ethiopia also documented that level of carcass contamination was considered as an outcome variable taking skin swab, mesenteric lymph node, cecal content, evisceration's hand swab, eviscerating knife swab and water samples *Salmonella* status and total slaughter volume as risk factors for carcass contamination [11].

The study conducted in Canada showed that independent variables, when tested individually, indicated that *Salmonella* contamination of scalding tanks, knives, and boots, cleanliness of hogs, and the number of chain stops was associated with the prevalence of *Salmonella* in the lots. No difference was found between clean lots and relatively clean ones. There was a positive, but not significant, correlation between the prevalence of *Salmonella* on carcasses and chain speed and the frequency of knife washing. There was no correlation between prevalence of *Salmonella* on carcasses and cleaning product concentration used: chlorine or quaternary ammonium. *Salmonella* prevalence was similar for the two types of cleaning products and for the two types of rinsing [52].

3. PREVALENCE OF *SALMONELLA* SPECIES

3.1. Global overview

In many countries incidence of human *Salmonella* infection has increased drastically over the years. The two most commonly isolated serotypes of concern and mostly implicated in disease outbreaks are *Salmonella enterica* serotype Typhimurium and *Salmonella enterica* serotype Enteritidis [64; 65; 66]. Besides the importance of this microorganism for public health, another aspect is the cost generated by human salmonellosis. During 1999, the cost linked to food borne salmonellosis ranged between 560 million and 2.8 billion € in Europe, where *Salmonella* was estimated to be responsible for nearly 166 000 cases [67].

It is reported that the rate of salmonellosis in the United States is between 15 to 20 cases per 100, 000 people [68]. The *Salmonella* species is one of the eight microorganisms in the European Union Zoonoses Monitoring Directive (EUZMD), which shows it is a disease considered to have a high impact on human and animal health in the Union [69]. The Enter-net surveillance program reported *Salmonella enterica* serotypes Enteritidis and Typhimurium, the most predominant organisms identified by the participating countries making up over 80% of all isolates during the period of 1998-2003. It also reported that for all *Salmonella* the general trend is declining with a reduction of 35.3% in 2003 over 1998 [70].

The burden of salmonellosis in Peshawar, Pakistan was estimated from published studies that Prevalence of *Salmonella* on cattle body coat, carcasses, slaughtering floor and tools of the butchers were investigated. The animals were divided into two groups i.e. washed and unwashed animals. *Salmonella* was found in 100% samples. Carcasses samples from unwashed animals had significantly higher log total viable count of *Salmonella* (24.45 ± 0.06) as compared with washed animals (21.77 ± 0.05) [71]. Studies in Spain showed that *Salmonella* was detected in 9 (17.3%) of the cattle samples. All of the isolates were characterized as *Salmonella enterica* serotype Frintrop [72]. In the United Arab Emirates however, Wernery [73] found the prevalence of *Salmonella* in camels to be less than five percent. Another study in United Arab Emirates on fresh chicken meat samples 46.67% was positive for *Salmonella* of the total samples. Samples obtained from the supermarkets tested negative for *Salmonella* while chicken samples obtained from the butcheries tested positive [74]. In Australia, *Salmonella* was detected in 21 (6.8%) of the 310 cattle tested, 14 (9%) from lot-fed cattle feces and seven (4.5%) from grass-fed cattle feces. There was no significant difference in the prevalence of *Salmonella* between grass fed and lot-fed cattle [75]. In Lao People's Democratic Republic the reported organism were isolated from cecum samples of buffaloes and pigs. The organisms were a prevalence of 8% (4/50) from buffaloes and 76% (37/49) from pigs. In buffaloes, 1 animal harbored both *S. Derby* and *S. Javiana*. In pigs, the most predominant serotypes were *S. Derby* (51%) followed by *S. Anatum* (45%), *S. Weltevreden* (15%) and *S. Stanley* (5%) [76].

In Bhutan, the prevalence of *Salmonella* was 13% with *Salmonella* Enteritidis as the most frequently isolated serotype (84.62%), followed by *Salmonella* Typhimurium (15.38%). The isolation of *Salmonella* during winter and late spring was significantly different. Broiler carcasses were 10.62 times more likely to yield *Salmonella* in the hot season as compared to the winter season [3]. The reported prevalence of *Salmonella* in South Asian countries varies from country to country. Studies in northern Thailand revealed 57% prevalence during 2002-2003 [77], 14.5% prevalence in Kathmandu, Nepal [78], and 42.63% prevalence in Vietnam [79]. Sero-prevalence of *Salmonella* in Bangladesh has been reported to be 23.46% [80]. Not much literature has been available on the prevalence of *Salmonella* in carcasses, few researches reports negligible [81] to as low as 5% [82], to a prevalence of 69% [83]. The overall annual incidence of food borne salmonellosis in India is nearly 6 per 1000 inhabitants [1]. In Korea a total of 5.28% *Salmonella* species was isolated from fecal materials and organ samples. The predominant *Salmonella* is *Salmonella enterica* serotype and serovar was group B (69.8%) and *Salmonella* Typhimurium (47.6%), *S. Derby* (20.6%) and *S. Heidelberg* (1.6%) [84].

3.2 Status in African countries

The Food and Agriculture Organization of the United Nations and the World Health Organization jointly state that "illness due to contaminated food was perhaps the most widespread health problem in the contemporary world," and "an important cause of reduced economic productivity" [85]. With the increasing population in the developing world, there is an increasing demand for meat and meat products which will force the present resource driven system of livestock production to a demand driven system [86] which will increase the disease transmission risks. There is a multi-factorial risk of food borne hazards in the developing countries due to poor sanitation and inadequate access to potable water [1].

Studies conducted in different regions of Africa including Namibia, Kenya and Nigeria have always topped the incidence of salmonellosis [87; 4; 88] and is the most seriously perceived food risks, even in the developed countries [89]. In Kenya sixteen (13.8%) of 116 samples were positive for *Salmonella*. Three *Salmonella enterica* subspecies *enterica* serovars, namely Saintpaul, Braenderup, and Heidelberg were identified, *S. Saintpaul* being predominant [4]. Also in Namibia from a total samples examined, 10.9% were found to be positive for *Salmonella*. A total of 29 *Salmonella* serovars were identified from one or both sample types, with *S. Chester* being the most frequent isolated, followed by *S. Schwarzengrund* and *S. Chartres* [88]. In Nigeria Lagos among *Salmonella* species isolated from the stool samples collected from food handlers were *S. Typhi*, *S. Enteritidis*, *S. Choleraesuis*, *S. ParatyphiA* and *S. Arizona* with prevalence of 6.8%, 5.3%, 2.9%, 1.5% and 0.5%, respectively. *S. Enteritidis* and *S. Typhimurium* were isolated from fecal cattle samples with prevalence of 12% and 3%, respectively [87].

Comment [sp12]: Over 2000 serotypes non typhoidal *Salmonella* are still identified. Country wise/ global effect of these should be included.

3.3. Status in Ethiopia

Like other developing countries, in Ethiopia *Salmonella* species are the major cause of food born disease and it cause mortality and morbidity particularly in human and animal. A cross-sectional study was conducted in central region of Ethiopia to determine the prevalence and distribution of *Salmonella* serotypes in minced meat beef, mutton and pork from retail supermarkets reported that out of the total meat samples examined, 44 (14.7%) were *Salmonella* positive. *Salmonella* was detected in 14.4% (23/160) minced beef, 14.1% (12/85) mutton and 16.4% (9/55) pork samples analyzed. Of the total 44 *Salmonella* positive samples, nine different serotypes were identified. The dominant serotype identified was *S. Infantis* (36.4%) followed by *S. Braenderup* (29.5%), *S. Anatum* (9.1%) and *S. Bovismorbificans* (9.1%). Other *Salmonella* serotypes isolated include *S. Vejle*, *S. Dublin*, *S. Saintpaul*, *S. Infantis* and *S. Braenderup* were isolated from minced beef, mutton and pork samples whereas *S. Dublin* and *S. Saintpaul* were isolated only from minced beef samples [18].

In recent time a study by FentabilGetnet [90] in the central part of Ethiopia (Addis Ababa) reported that eight *Salmonella* species were isolated among 233 food handlers giving an isolation rate of 3.4%, all were females. Of these; two *S. Typhi*, one *S. ParatyphiA* and five unidentified *Salmonella* species were isolated. A study conducted by BayleyegnMolla *et al.* [7] different serotypes were identified from slaughtered cattle (4.2%), camels (16.2%), chicken meat and giblets (23.6%). Among this predominant serovars were *S. Braenderup*, *S. Dublin* and *S. Saintpaul* followed by *S. Typhimurium* (including var. Copenhagen) and *S. Anatum*. Similarly, a study in Addis Ababa from September 2003 to February 2004 documented that *Salmonella* species isolated from food items and stool samples, of which, 7.8% were positive for *Salmonella* from food samples and of sixty-eight stool samples five gave positive result (7.4%). About 14% of chicken carcass, 11.3% of pork, 10.8% of mutton, 8.5% of minced beef, 2.1% of cottage cheese, 2.3% of fish and none of the ice cream yielded *Salmonella*. A total of 14 different serotypes out of 98 *Salmonella* isolates were identified. *Salmonella* Newport (41.8%) was the most prevalent serotype, followed by *S. Braenderup* (12.2%), *S. Hadar* (8.2%), *S. Typhimurium* (7.1%), *S. Dublin* (6.1%) and *S. Haifa* (6.1%). Less commonly isolated *Salmonella* serotypes included: *S. Infantis*, *S. Kentucky*, *S. Bovismorbificans*, *S. Anatum*, *S. Zanzibar*, *S. Kottbus*, *S. Saintpaul* and *Salmonella* Newport and *S. Kentucky* were reported for the first time in Ethiopia. *Salmonella* Newport was isolated from all sample types except ice cream, while *S. Braenderup*, *S. Kottbus*, *S. Saintpaul* were detected only from chicken carcass, pork and minced beef samples, respectively [17].

In Bahir Dar *Salmonella* isolates from cattle consisting of *Salmonella* Typhimurium, *Salmonella* Newport, *Salmonella* Haifa, *Salmonella* Heidelberg, *Salmonella* Infantis, and *Salmonella* Mishmarhaemek were identified. *Salmonella* Typhimurium and *Salmonella* Newport were most frequently isolated while *Salmonella* Heidelberg and *Salmonella* Mishmarhaemek were isolated least [91]. According to Sefinew Alemu and BayleyegnMolla [91] *Salmonella* was detected from liver, mesenteric lymph nodes, carcass swab, and intestinal content samples with prevalence of 1.1%, 3.2%, 4.8%, and 5.9%, respectively. According to BayehAbera *et al.* [92] 1.6% food handler were found positive for *S. Typhi*. Of these, 6.5% were suffering from diarrhea.

4. ANTIMICROBIAL SUSCEPTIBILITY OF SALMONELLA

Until recently, *Salmonella* species were highly susceptible to the most commonly used antibiotics [93]. The resistance of *Salmonella* to a single antibiotic was first reported in the early 1960s [94; 34]. The most widely used antibiotics for treatment of salmonellosis in humans is a group of fluoroquinolones and third-generation cephalosporins. The earlier drugs chloramphenicol, ampicillin, amoxicillin and trimethoprim-sulfamethoxazole are occasionally used as alternatives [95]. Since then, the isolation frequency of *Salmonella* strains resistant to one or more antibiotics have increased in the Saudi Arabia, United States, United Kingdom and other countries of the world. This is due to the increased and uncontrolled use as well as easy accessibility to antibiotics in many countries of the world [96; 34]. Emerging resistance in *Salmonella* has been described especially in Africa and Asia and the appearance of *Salmonella* Typhimurium DT104 in the late 1980s raised main public health concern, thereby threatening the lives of infected individuals stated that multi-resistance occurred in *Salmonella* species [97; 94; 34].

Fluoroquinolones are the most commonly used antimicrobial agent for the treatment of invasive *Salmonella* infections in adults [98]. *Salmonella* isolates with decreased susceptibility to fluoroquinolones (but that are not resistant to fluoroquinolones) commonly have a single point mutation in a chromosomal gene [99]. Third-generation cephalosporins, such as ceftriaxone, are commonly used for treatment of invasive *Salmonella* infections in children because of their pharmacodynamic properties and low prevalence of resistance to these agents. Therefore, there is concern about the potential emergence of ceftriaxone-resistant *Salmonella*. The first reported case of domestically acquired ceftriaxone-resistant *Salmonella* was in a 12-year-old child in Nebraska [100].

High degree of multiple drug resistance was observed in *Salmonella* isolates from different food animal samples that indicate drug resistance of *Salmonella* is becoming a crucial health problem in this part of the world. The prevalence of *Salmonella* strains resistant to more than one antibiotic may be due to the comprehensive use of antibiotics included in feeds as growth promoters and due to the widespread use of antibiotics in food animal industries. Studies in Dubai, United Arab Emirates reported that all the isolates (100%) showed resistance to cephalixin and rifampicin. A high degree of resistance was also observed for ampicillin and tetracycline while 87.88% of these isolates were sensitive to ciprofloxacin

Comment [sp13]: Water borne diseases due to *Salmonella* infection should also be included.

Comment [sp14]: MECHANISM MUST BE DISCUSSED
"Resistance in bacterial population can be spread from person to person/ animal to animal/ animal to human by bacteria, from bacterium to bacterium by plasmids, from plasmid to plasmid or chromosome by transposons"
Discussion on these points MUST be included.

and amikacin [74]. A cross-sectional study was conducted from November 2006 to April 2007 in Bhutan was reported the antimicrobial susceptibility of *Salmonella* isolates. Among seven antimicrobial tested, resistance was highest for nalidixic acid (96.15%) followed by amoxicillin (11.54%) and cephalexin (5.77%). Ciprofloxacin and sulpha-trimethoprim showed resistance of 1.92% each. While gentamicin was sensitive to all the isolates tested, chloramphenicol had a sensitivity of 98.08%. The isolates were resistant to a maximum of three antimicrobials. All eight *Salmonella* Typhimurium isolates were resistant to nalidixic acid with one isolate showing simultaneous resistance to cephalexin. *Salmonella* Enteritidis was resistant to five of the seven antimicrobials tested with simultaneous multidrug resistance in up to three antimicrobials [3].

In Korea antimicrobial susceptibility of the *Salmonella* isolated from pig varies as follows: norfloxacin (75%), ciprofloxacin (67.5%), amikacin (60%), colistin (60%), enrofloxacin (55%). All of isolates were resistant to erythromycin, penicillin, tetracycline and lincomycin [84]. In Tehran, Iran, among the variety of antibiotics tested, the highest resistance was found with nalidixic acid followed by tetracycline, trimethoprim, and streptomycin. The percentages resistance of isolates from meat samples was 36.8%, 21%, 26.3%, and 5.3%, respectively. About 23.5% of the *Salmonella* strains were multi-resistant to two or more antibiotic families. In overall, the degree of resistance of *Salmonella* to nalidixic acid was greater than other tested antibiotics [101].

In USA study conducted on cattle carcass and feces showed that *Salmonella* isolates were tested for antimicrobial drug susceptibility. Among this 97% ($n = 101$) of the isolates were resistant to at least one antimicrobial drug; however, only 4.0% were resistant to two or more. The most common resistance was to sulfamethoxazole. These results indicate that the presence of microorganisms resistant to antimicrobial drugs is common in cattle and beef [102]. *Salmonella* isolates recovered from dairy cows had relatively little resistance to the antimicrobial agents; 83.0% of the isolates were susceptible to all antimicrobials tested [29]. Another study in USA indicated that of the 18 *Salmonella*-positive lymph node samples, 3 contained multidrug-resistant *Salmonella*. All three of these samples were from lymph nodes removed from the carcasses of cull cattle [103].

In Brazil *Salmonella* isolates from broiler carcasses observed resistance to colistin, novobiocin, erythromycin and tetracycline in 100% isolates. Strains showed intermediate resistance at different levels to kanamycin (1.25%), enrofloxacin (3.75%), neomycin (3.75%), fosfomicin (20%), sulphonamides (86.25%) and nitrofurantion (90%). Resistance to ciprofloxacin, norfloxacin, gentamicin, polymyxin B, sulphametrim and sulphazotrim was not found [104]. In Canada a study conducted in swine farm reported that more than half of the isolates (53.4%) were susceptible to all of the 18 antimicrobials. No resistance was observed to amikacin, amoxicillin/clavulanic acid, cefoxitin, ceftiofur, ceftriaxone, cephalothin, ciprofloxacin, imipenem or nalidixic acid. Less than 1% of isolates were resistant to apramycin, gentamicin and trimethoprim/sulfamethoxazole. Higher frequencies of resistance were observed for chloramphenicol (4.7%), ampicillin (7.8%), kanamycin (11.8%), sulfamethoxazole (21.1%), streptomycin (25.5%) and tetracycline (38.8%) [105]. Study in Rockville, Maryland showed that of the 257 *Salmonella* isolates obtained, 54 isolates (21%) were resistant to at least one antimicrobial [106].

Studies in European countries indicated that *Salmonella* were isolated from food producing animals and tested for antimicrobial susceptibility. In Japan *Salmonella* isolates resistant to ampicillin, dihydrostreptomycin, kanamycin, oxytetracycline, chloramphenicol, bicozamycin, nalidixic acid, oxolinic acid and trimethoprim were obtained from healthy animals and diagnostic sample submissions. *Salmonella* Dublin was isolated only from cattle and showed resistance to older quinolones [107]. Another study in Japan on food producing animal was indicated that resistance was found for 8 of 11 antimicrobials tested, at the following rates: 46.4% for dihydrostreptomycin followed by ampicillin and oxytetracycline (both 8.9%) [108]. In Faisalabad, Pakistan also *Salmonella* isolates showed 100% resistance against bacitracin, erythromycin and novobiocin [109]. Similarly in Canary Islands, Spain all isolates were susceptible to all of the tested antimicrobial agents, which included ampicillin, amoxicillin/ clavulanic acid, tetracycline, enrofloxacin, chloramphenicol, nalidixic acid, piperacillin and trimethoprim-sulfamethoxazole [72]. Another study in Spain also showed that all isolates were multi-resistant. The average number of resistances per strain increased from 3.98 in 1993 to 5.00 in 2006. An increase in the incidence of resistance was observed between 1993 and 2006 for cephalothin, enrofloxacin and tetracycline [110].

Resistance in *Salmonella* has been described especially in Africa countries and the appearance of *Salmonella* Typhimurium DT104 in the late 1980s raised main public health concern [97; 94; 34]. In Namibia study conducted in food animals documented that from the *Salmonella* isolates, 19.7% were resistance to one or more of the antimicrobials whereas 80.3% were susceptible to all 16 antimicrobials tested. Resistance to sulfoxazole and the trimethoprim-sulfamethoxazole combination were the most common [88]. In Sudan, 46.8% of *Salmonella* serotypes isolated from animal were found to be resistant to at least one of the tested nine antimicrobial agents and 45 isolates (37.8%) were found to be multidrug-resistant. Ciprofloxacin and gentamicin were found to be highly active against the isolates. But the isolates showed high resistance to ampicillin, chloramphenicol, tetracycline and sulfamethoxazole-trimethoprim [111]. According to Fasureet *et al.* [112] reported in Nigeria *Salmonella* isolates were 100% resistant to ampicillin, 90.6% to tetracycline and moderately sensitive to nalidixic acid (62.5%). Fluoroquinolone resistant *S. Typhimurium* strains from food animal were also observed. In Meknès, Morocco, 43 (75.43%) *Salmonella* isolates were resistant to one or more antimicrobials. Out of 43 resistant *Salmonella* isolates, 17 (39.5%) showed multiple resistance to two or more different antimicrobials. Resistance to tetracycline, sulfamides, trimethoprim and streptomycin was the most frequent [113]. According to Gideon *et al.* [4] in Kenya, antimicrobial resistance was found in 35.7% of the isolates. The *S. Heidelberg*

isolates were susceptible to all the antimicrobials tested. Multidrug-resistance was found in 7.1% of the *Salmonella* isolates.

Salmonella isolates from Ethiopia at different times showed that *Salmonella* were susceptible to ciprofloxacin, cotrimoxazole, and chloramphenicol [2], resistance to streptomycin (24/32, 75%), ampicillin (19/32, 59.4%), tetracycline (15/32, 46.9%), spectinomycin (13/32, 40.6%) and sulfisoxazole (13/32, 40.6%) [17]. Studies conducted in Addis Ababa documented that the antimicrobial susceptibility profile showed all except one were resistant to ampicillin and all isolates were resistant at least to one of antimicrobials tested [90]. Antimicrobial resistance was most common among *Salmonella* isolated from carcass (18/29, 62.1%) followed by pork (5/22, 22.7%). Multiple antimicrobial drug resistance was observed in 23 *Salmonella* isolates (23.5 %) [17]. Eleven of the 28 (39.3%) *Salmonella* isolate from cattle were resistant to one or more of the antimicrobials tested. Resistance was shown to ampicillin, chloramphenicol, gentamycin, norfloxacin, polymyxin-B, streptomycin, tetracycline, and trimethoprim. Four of 11 (36.4%) were multiple antimicrobial resistant. All the isolates tested were susceptible to the antimicrobial effects of gentamycin, norfloxacin, and trimethoprim. Eleven, four, and two isolates of the 28 were resistant to streptomycin, tetracycline, and ampicillin, respectively [91]. According to BayleyegnMolla *et al.* [7], fifty-one (63.7%) of the 80 *Salmonella* strains were resistant to one or more antimicrobials of which 42 (52.5%) displayed multiple-drug resistance. Among the strains, 51.2% were resistant to sulfisoxazole, 46.2% to spectinomycin, 45% to amoxicillin-clavulanic acid and ampicillin, 41.2% to tetracycline and 30% to chloramphenicol.

5. CONCLUSION

Problems have their origin in the methods of farming of animal foods. Many farmers are illiterate and follow methods of production that are centuries old. They live in very close contact with their animals, often under poor hygienic conditions, thereby increasing the likelihood of food borne zoonoses. However, considerable proportion of patients may not visit health centers unless symptoms are serious due to shortage of resources and lack of awareness. Both single and multiple antimicrobial resistance patterns to the commonly practiced antimicrobials in the veterinary and public health set up were observed, which is of special concern in developing country where use of antimicrobials has problems. In animals, there is treatment restriction because of inadequate drug alternatives; therefore, limited drugs are frequently used for treatment; this practice leads resistance to limited antibiotics. In addition to this, multidrug resistance was observed due to lack of restricting, discriminate and appropriate use of antibiotics in the food animal industry.

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Comment [sp15]: After finalising the article, cross check the references of the text with the reference list and vice versa.

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